Enhanced isolation of adult thymic epithelial cell subsets for multiparameter flow cytometry and gene expression analysis.

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Public Summary:
The epithelial cells (TECs) are microenvironmental niche cells which support T lymphocyte development in the thymus. Most studies of TEC biology have focused on TEC at the fetal stage of development, whereas the biology of adult-stage TECs is not as well-understood. Delineating the molecular mechanisms that control adult TEC differentiation has implications for the success of T-lymphocyte based therapies for autoimmune diseases and induction of immunological tolerance to stem cell-derived tissues. Here, we have devised an improved isolation protocol for adult mouse TECs and combined it with six-color multiparameter flow cytometry. Using these techniques, we have identified four distinct subsets of CD45- EpCAM+ TECs in adult mice, and PCR analysis verified that these TEC subsets differentially expressed known TEC genes. TEC subsets were further analyzed using high-throughput quantitative PCR arrays to reveal novel genes that could be important for TEC subset maintenance. Our enhanced isolation allows for detailed analysis of rare TEC subpopulations in the adult mouse at the cellular and molecular levels.

Scientific Abstract:
The epithelial cells (TECs) are microenvironmental niche cells which support T lymphocyte development in the thymus. Most studies of TEC biology have focused on TEC at the fetal stage of development, whereas the biology of adult-stage TECs is not as well-understood. Delineating the molecular mechanisms that control adult TEC differentiation has implications for the success of T-lymphocyte based therapies for autoimmune diseases and induction of immunological tolerance to stem cell-derived tissues. Detailed analysis of adult TECs is technically challenging due to their rarity, their diminishing numbers with age, and the limited number of markers to distinguish between unique TEC subpopulations. Here, we have devised an improved isolation protocol for adult mouse TECs and combined it with six-color multiparameter flow cytometry. Using these techniques, we have identified four distinct subsets of CD45- EpCAM+ TECs in adult mice: a) UEA1(low) CDR1(low) (UC(low)); b) UEA1(high) CDR1(high)(UC(high)); c) UEA1(low) CDR1(high) MHC(high) (cTEC); and d) UEA1(high)CDR1(low) MHC(int/high) (mTEC). PCR analysis verified that these TEC subsets differentially expressed known TEC genes. TEC subsets were further analyzed using high-throughput quantitative PCR arrays to reveal novel genes that could be important for TEC subset maintenance. Intracellular staining for keratin-5 and keratin-8 can also be added, but our results suggest that keratin expression alone cannot be used to distinguish adult TEC subsets. Our enhanced isolation allows for detailed analysis of rare TEC subpopulations in the adult mouse at the cellular and molecular levels.